

**Background.** WET tests are conducted by subjecting organisms to various concentrations of an effluent in a controlled laboratory setting. In most cases, the actual receiving water is used as the diluent in these tests in order to provide as realistic a simulation of the discharge situation as possible. WET tests required by the State of Maine are conducted to determine the No Observed Effect Level (NOEL) for both acute and chronic conditions. The acute end point is survival of the test organisms over a 24-96 hour time period. The chronic end point is evaluated on the basis of the growth or reproduction of the test organism over a usually longer period of time. The results of the tests are expressed as the percent effluent that does not cause an effect on the test organisms when compared statistically to receiving water and laboratory controls. The standard for compliance, or *receiving water concentration*, is expressed as a percentage of the relevant dilution factor (acute or chronic) divided in to 100 percent. For fresh water discharges, a reduced dilution factor is needed for facilities that do not receive good initial mixing due to the location or the outfall pipe, such as on the bank of the river, rather than extended into the flow of the current. In these situations, one quarter of the acute dilution factor (based on the 1Q10 flow) is used; this is termed the  $\frac{1}{4}$ 1Q10 dilution. Unless the permittee has provided information regarding the placement of its outfall, the DEP assumes the effluent is not well mixed and does apply the  $\frac{1}{4}$ 1Q10 dilution factor for analysis of test results.

The organisms used for testing of fresh water discharges include water fleas and either fathead minnows or brook trout. The selection of which vertebrate species is controlled by the provisions of Chapter 530.5 and EPA requirements. At times of the year, Brook Trout may not be available in the proper size and age and with DEP concurrence Rainbow Trout may be substituted. Both acute and chronic tests are conducted on all species.

The organisms used for testing of marine discharges include sea urchin (chronic only), mysid shrimp (acute only) and atlantic silverside (acute and chronic).

In addition to NOEL testing required by DEP, the EPA has required WET testing as well. This testing is often done as LC50, an acute test that measures the effluent concentration that results in 50% mortality of the test organisms. While the results of these tests are reported to DEP and maintained in the Department's database, they have not been included in the evaluation of data presented here. However, in conducting LC50 tests, it is possible to also determine an acute NOEL value, and the Department encourages submission of this information, even if not necessary to meet concurrent NOEL test requirements for DEP.

**Overview of WET test data.** Through, mid-May 2001, some 121 discharge sources have conducted WET testing. A number of these sources are not currently subject to Chapter 530.5. Some are now closed or did testing for other purposes such as remediation of hazardous materials spills. Much of the data from such sources date back to the early to mid 1990's, but have been included in the Department's data analysis to provide the most robust review possible. For some purposes, test data have been considered in two ways: all information and those tests conducted within the past five years. Considering all of the data allows the broadest consideration in trends, while the most recent five years provides a picture of more current compliance trends. For regulatory purposes, the Department relies on the past five years since

that time is typically the renewal cycle for most permits. In total, there are approximately 3,020 acute NOEL test results in DEP database, with about 2000 having been done since January 1, 1996. The database contains over 2,500 chronic NOEL tests, with approximately 1,550 having been done since January of 1996. Table I summarize all the NOEL tests by species.

Table I. Distribution of NOEL WET tests by species - all data

	Chronic Results	Acute Results
Fresh Water		
Water Flea	886	1077
Trout	389	430
Fathead Minnow	376	479
Marine Species		
Silversides	424	515
Sea Urchin	458	---
Mysid Shrimp	---	518

The numbers of individual species tests differ due to repeated tests and testing to meet EPA requirements. Because EPA has used LC50 tests, the number of acute NOEL results available to DEP is increased. These tests include results reported as a less than (" $<$ ") value. Such reports represent approximately 1.3% of the total number of tests. In recent years, the Department has made a point of specifying that test dilutions be set so as to bracket the discharge source's receiving water concentrations in order to obtain a definitive determination as to compliance with water quality requirements. Table II presents the tests done by year, along with the number of less than values reported.

Table II. Distribution of NOEL WET tests by year - all data

	Chronic Tests		Acute Tests	
Year	Number	$<$ values	Number	$<$ values
Pre-1994	404	16	387	2
1994	239	8	220	2
1995	324	5	412	2
1996	291	7	412	1
1997	290	12	408	1
1998	312	6	390	0
1999	338	4	401	0
2000	292	5	341	0
Totals	2,490	63	2,971	8

As a final presentation of the universe of all the WET data, Table III shows the tests arranged by numeric results. The lower the percentage of an individual result, the greater degree of absolute toxicity an effluent has. However, the actual toxicity in a receiving water must also include consideration of the dilution the effluent receives. Nonetheless, the absolute toxicity of effluents can be useful in evaluating the general performance of the toxicity program.

Table III. Distribution of all NOEL WET Test Results

	Fathead		Trout		Water Flea		Silverside		Shrimp	Urchin
	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Reported as "<"	0	5	0	6	5	27	1	4	2	20
Under 10%	0	6	2	14	8	62	7	10	24	52
10% to 24%	13	17	6	14	14	81	8	18	26	37
25% to 49%	26	27	11	48	58	165	15	26	61	58
50% to 74%	43	53	32	72	80	205	65	64	63	113
75% to 99%	6	1	5	1	9	2	75	44	79	13
100%	391	267	374	234	903	344	344	258	263	165
Total Tests	479	376	430	389	1077	886	515	424	518	458

Table IV. Distribution of NOEL Exceedences

	Data for Tests Since January 1, 1996					Data for All Tests on File				
	Total tests	Exceedences		Reported as "<"		Total tests	Exceedences		Reported as "<"	
<b>Chronic Tests</b>	1,556	58	3.7%	15	1.0%	2,533	103	4.1%	26	1.0%
Water Fleas	495	30	6.1%	4	0.8%	886	64	7.2%	7	0.8%
Trout	235	10	4.3%	1	0.4%	389	14	3.6%	2	0.5%
Fatheads	224	3	1.3%	2	0.9%	376	4	1.1%	2	0.5%
Silversides	289	1	0.3%	2	0.7%	424	2	0.5%	3	0.7%
Urchins	313	14	4.5%	6	1.9%	458	19	4.1%	12	2.6%
<b>Acute Tests</b>	2,001	17	0.8%	0	0.0%	3,019	42	1.4%	7	0.2%
Water Fleas	636	1	0.2%	0	0.0%	1077	18	1.7%	5	0.5%
Trout	295	0	0.0%	0	0.0%	430	3	0.7%	0	0.0%
Fatheads	312	0	0.0%	0	0.0%	479	1	0.2%	0	0.0%
Silversides	377	5	1.3%	0	0.0%	515	6	1.2%	0	0.0%
Shrimp	381	11	2.9%	0	0.0%	518	14	2.7%	2	0.4%

Exceedences includes less than values below the facility's critical level

Reported "<" includes less than values reported above the facility's critical level

**Review of WET exceedences.** Exceedences represent instances where the reported test value of a WET test is below the critical percentage. As noted above, a small number of test results are reported as less than. Since these test values are at the low end of the spectrum, they are more likely to represent exceedences and accordingly deserve careful consideration. In some cases, a WET test may be at a level below the facility's receiving water concentration. In these situations, the result is obviously an exceedence. For example, a facility may have a receiving water concentration of 5.0% and the reported test result is <3.5%. However, some results have been reported as less than a value higher than the receiving water concentration. That is, the facility's value is 5.0% and the reported result is <8.5%. In this situation, it is not clear if the actual level of toxicity, had it been fully defined, would have been an exceedence or not. Of the 71 test results reported as less than, 38 were at levels below the critical level for the respective facility. Conversely, the remaining 33 reported less than values above the receiving water concentration. The data tables break out the less than values on the basis of whether they were above the critical level.

In Table IV, the exceedences are summarized. For fresh water species, the acute tests include both facilities with good effluent mixing and those where the 1/41Q10 dilution applies. The table presents information for all tests on file and those done since January 1, 1996. A separate column shows the less than values reported above the respective facilities' critical levels for each test species. Less than values reported above critical levels were more prevalent with chronic NOEL (1.0% of the tests) than for acute (0.2% of the tests). Chronic tests are more likely to incur exceedences than are acute tests, with 4.1 % of all chronic tests and 1.4% of all acute tests in exceedence. For the most recent five years, the exceedence rates are lower, with chronic at 3.7% and acute at 0.8%. These rates are for the number of individual tests, not the number of facilities.

For fresh water species, water fleas and trout chronic tests account for the majority of the exceedences detected. In the past five years, acute testing for fresh water species has identified 43 chronic exceedences in 954 tests (4.5%) and only one acute exceedence in 1243 tests (0.08%).

For marine species represented in Table IV, sea urchin tests account for all but one exceedence in 602 chronic reports since January 1, 1996, and the overall exceedence rate was about 2.5%. Acute marine testing reported 16 exceedences in 758 tests (2.1%). Mysid shrimp were the more sensitive than silversides in detecting acute exceedences.

The most important consideration for evaluating the occurrence of exceedences is the facility's dilution factor. Table V presents the exceedences by the dilution factors for the respective facilities, including the 1/41Q10 dilution for acute tests where it is applicable. The table represents all NOEL tests on file, including less than values below the respective critical levels. Less than values above the respective critical levels are not included in this table. For the chronic tests, with one exception all exceedences above a dilution factor of 50:1 occurred at industrial facilities. With the exception of one industrial facility that has had a history of very low test results, all acute exceedences have been reported by facilities having dilution factors below 25:1, and mostly below 10:1. Facilities that record exceedences are likely to have multiple occurrences. In most cases, this appears to be more attributable to a lower dilution ratio than particularly low test results.

Table V. Distribution of NOEL exceedences by facility dilution factor - all data

Dilution	Chronic Tests		Acute Tests		Acute Tests @ 1/41Q10	
	Exceedences	Facilities	Exceedences	Facilities	Exceedences	Facilities
< 5:1	32	8	10	4	7	3
5:1 - 10:1	12	4	3	3	11	1
11:1 - 15:1	9	4	2	1	1	1
16:1 - 20:1	25	6	0	0	0	0
21:1 - 30:1	2	1	2	1	0	0
31:1 - 40:1	13	3	0	0	0	0
41:1 - 50:1	2	1	0	0	0	0
51:1 - 100:1	2	2	0	0	0	0
>100:1	6	3	6	1	0	0
Totals	103	32	23	10	18	4

**Review of Reasonable Potential determinations.** Reasonable potential is always associated with an exceedence. But it may also occur when there is no exceedence if the minimum test result in the data set is relatively low in comparison to the facility's dilution factor. Of the 107 discharge sources having reported NOEL WET results for the period since January 1, 1996, 38 demonstrated reasonable potential for one or more species. There were a total of 53 test-species among these facilities where reasonable potential was found without a corresponding exceedence. In some cases, more than one test could have been low enough to cause reasonable potential for a given species at a facility, but this aspect of the database was not investigated. Of the 38 facilities with reasonable potential, some had more than one species in reasonable potential or exceedences for species other than the one(s) causing reasonable potential. However, 27 facilities demonstrated reasonable potential without any exceedences. Reasonable potential was found at eight facilities and a total of 12 species at the 1/41Q10 dilution where an evaluation using only the regular acute dilution did not find reasonable potential.

Because reasonable potential is a statistical evaluation, there are some practical limitations that should be considered in the use of these determinations. If a facility has conducted a relatively small number of tests and/or its dilution factor is low, reasonable potential may exist no matter what the actual test values may be. In the data set since January 1, 1996, a total of 10 species were in reasonable potential when the lowest result was 50% or greater and 2 or more tests had conducted. A total of 24 individual tests in this group were reported at 100%. When a facility's dilution factor is below 6:1, reasonable potential will be found irrespective of the actual test result. Table VI shows the minimum test result that can be recorded without a facility being placed in reasonable potential at various numbers of tests having been done.

Table VI. Minimum test result in % needed to avoid reasonable potential

Tests done	Dilution factor						
	1:1	2:1	3:1	4:1	5:1	6:1	7:1
1	>100	>100	>100	>100	>100	>100	87
2	>100	>100	>100	95	76	63	54
3	>100	>100	100	75	60	50	43
4	>100	>100	87	65	52	43	37
5	>100	>100	76	58	46	38	33
6	>100	>100	70	53	42	35	30
7	>100	100	67	50	40	33	29
8	>100	95	63	48	38	32	27
9	>100	90	60	45	36	30	26

Conversely, a facility with a large dilution factor may have only a remote possibility of being placed in reasonable potential. To illustrate this, Table VII. shows the dilution factors above which reasonable potential cannot be found with an effluent NOEL of 1% and a given various numbers of tests.

Table VII. Dilution factors needed to avoid reasonable potential with NOEL of 1%

Number of tests done	Minimum dilution factor to avoid reasonable potential at 1% NOEL
1	620
2	380
3	300
4	260
5	230
6	210
7	200
8	190
9	180